# INACTIVATION OF AFLATOXINS IN CONTAMINATED PEANUTS

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SUMMARY. – A highly contaminated peanut sample was treated to inactivate its toxicity by dry heating (roasting) or autoclaving. Aflatoxins of contaminated peanuts proved to be resistant to dry heating, while autoclaving was satisfactory in their destruction. Thin Layer Chromatography, toxicity to chick embryo and inhibition of *Bacillus megaterium* NRRL-B-1368 growth were used to evaluate the inactivation. It is recommended that after treatment, that crushed peanuts should be spray-dried before utilising.

RÉSUMÉ. – Deux méthodes ont été utilisées pour la décontamination des arachides : la torréfaction, l'autoclavage. La seconde méthode s'est montrée très efficace, contrairement à la première, pour détruire les aflatoxines. Les méthodes utilisées pour évaluer l'inactivation des aflatoxines sont la chromatographie sur plaques, la contamination de l'embryon de poulet et l'injection dans des colonies de *Bacillus megaterium* NRRL-B-1368. Après le traitement par autoclave, les arachides broyées doivent être séchées par soufflerie, avant leur utilisation.

## I. - INTRODUCTION

Grain and seed crops may be subject under certain unfavourable conditions to the attack of fungi which produces substances (mycotoxins) deleterious to animals and presumably to man as well. It is now clearly established that *Aspergillus flavus* grows on peanuts producing highly toxic compounds (aflatoxins) when they are stored after harvest without adequate drying (MAJUM-

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DER et al., 1965). The frequent occurence of aflatoxins in peanuts poses a serious problem in the utilization of peanuts products as a source of proteins diets.

Different methods have been suggested to remove or destroy toxins present in toxic peanuts. Numerous heat treatments for inactivation of aflatoxins have been proposed, but the reported results are contradictory. BLOUNT (1961) indicates that heating ground nut meals at 150°C for one hour makes this product more toxic for turkeys. ALLCROFT et al. (1961) reported that toxicity of the meals to ducklings was always less after it had been heated. AUSTWICK & AYERST (1963) proved that steam heating or other processes do not inactive the toxins. On the other hand GOLDBLATT (1967) has shown that moist heat treatment of partially purified aflatoxins at 170°C under pressure of 80 to 90 psi for 10 to 20 mn leads to their destruction. For LEE et al. (1969) an over-all reduction of the different aflatoxin components ranging from 62-65 % and 67-69% obtained by oil or dry roasting respectively.

Egypt has a good market for peanuts and peanut products. A work concerning peanuts aflatoxins is this of considerable importance. The present investigation was undertaken to assess how much inactivation of aflatoxins contents in peanuts could be achieved by dry or wet heating.

## II. - MATERIALS AND METHODS

### Extraction of peanuts

Aflatoxin components of peanuts were extracted by the method applied at the Division of Food Chemistry and Technology, Food and Drug Administration, Washington (D.C.) (YIN, 1969). 50 g of peanuts with 200 ml of acetonitrile-water (9:1, v/v) and 100 ml hexane were homogenized for 5 mn in a blender at 16000 rpm. Liquid was recovered by decanting through fluted filter paper, collected in separatory funnel. 40 ml of acetonitrile-water layer (lower) were removed and evaporated under vacuum to dryness.

### Analysis

Qualitative as well as quantitative estimation of the different aflatoxins were achieved on thin layer chromatograms using silica gel G (Merck). The glass plate (20 x 20 cm) was covered with a 0.3 mm thick layer of silica gel. The residue of acetonitrile extract was diluted to 1 ml with chloroform and 0,05 ml of the solution applied to the plate. A standard of known composition was run on each chromatogram for qualitative and quantitative purposes. The plates were developed with chloroform/methanol (96:4, v/v) and the aflatoxins detected by visual examination of chromatograms under long UV light (365 mμ), where they appear intensely fluorescent (MOUBASHER et al., 1974 and NESHEIM, 1969).

### Toxicity Assay

Chick embryo and microbiological methods were used to provide evidence of detoxification of peanuts extracts :

1. — **Chick embryo method** : the technique of chicken egg air sac inoculation was used (VERRETT et al., 1964). A group of 5 fertile white Leghorn eggs was inoculated before incubation either with 0.02 ml of the concentrate or with various dilutions of the test materials. Dilutions of the extract were made with chloroform to determine the level of toxicity. Control eggs were treated with uninfected peanut extract in the same manner.

2. — **Microbiological method** : This assay technique is based on growth inhibition and cell abnormalities of *Bacillus megaterium* induced by aflatoxins (CLEMENTS, 1968 and JANICKI et al., 1975). 0.05 ml of the concentrate extract (or various dilutions) was applied to sterile filter paper disk 6 mm in diameter. After the chloroform had completely evaporated, the disks were placed on *B. megaterium* grown on tryptone-glucose-yeast extract (TGY) agar plates. The plates were then incubated at 35°C for 24 h., and the zone of growth inhibition measured.

## III. - RESULTS AND DISCUSSION

Trials were made to inactivate aflatoxins of a selected naturally contaminated crushed peanuts with aflatoxin content equal to 1900 $\mu$g/kg. The contaminated peanuts were either roasted 60, 80 and 105°C at different time intervals (1, 2 and 3 h), or autoclaving at 1.5 atm. for 30, 60 and 90 mn.

### Roasting

The data of TLC* analysis (Tab. 1) showed that roasting of crushed peanuts at 60°C did not appreciably affect total aflatoxin contents. At 80°C a little reduction (about 7% for aflatoxin $B_1$ and $G_1$) was recorded when peanuts were roasted for one hour. This effect was enhanced by increasing the heating period at the same temperature. At 105°C, the destruction was enhanced by increasing the time of heating. A reduction of about 60% of the total aflatoxin contents or 70% of $B_1$ and $G_1$ was obtained. Toxicity to chick embryos confirms TLC analysis. The untreated contaminated peanut extract exhibited 50% hatchability with 60-fold dilution of the concentrate extract. The toxicity gradually decreased with the increase of heating period at the different temperatures tested. The roasted sample for 3 h at 105°C yielded 50% hatchability with 30-fold dilution. Microbiological assay also indicated a reduction of aflatoxin contents of the treated peanuts compared with the untreated sample (Tab. 3).

* Thin layer chromatography.

| Aflatoxin Components (ug/kg) | Untreated sample | 60° C | | | 80° C | | | 105° C | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Time in hours | | | | | | | | |
| | | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| $B_1$ | 760 | 760 | 760 | 740 | 500 | 420 | 380 | 420 | 380 | 220 |
| $B_2$ | 260 | 260 | 260 | 260 | 260 | 250 | 230 | 240 | 230 | 220 |
| $G_1$ | 700 | 700 | 700 | 680 | 450 | 400 | 320 | 400 | 340 | 200 |
| $G_2$ | 180 | 180 | 180 | 180 | 170 | 170 | 160 | 170 | 160 | 140 |

Table 1. – Aflatoxin components ($\mu$ g/kg sample) as affected by roasting at different temperatures and several time intervals.

| Aflatoxin Components (ug/kg) | Untreated sample | Time (mn) | | |
|---|---|---|---|---|
| | | 30 | 60 | 90 |
| $B_1$ | 760 | 140 | ND | ND* |
| $B_2$ | 260 | 160 | 40 | ND |
| $G_1$ | 700 | 120 | ND | ND |
| $G_2$ | 180 | 120 | 30 | ND |

*ND = not detected.

Table 2. – Aflatoxin components ($\mu$g/kg sample) as affected by autoclaving at 1,5 atmosphere for different time intervals.

## Autoclaving

The results of these studies (Tab. 2) showed that autoclaving was effective in inactivating aflatoxins; none could be detected after treatment for 90 mn at 1.5 atm. These results are in harmony with the findings of MANN et al. (1967), who recorded that about 80% reduction in aflatoxins could be achieved by heating for 2 h at 100°C at 20% moisture. Treatment for 30 mn at 1,5 atm. reduced markedly the contents of $B_1$ and $G_1$ components (38 and 33% respectively). These results indicate that $B_2$ and $G_2$ are more resistant to autoclaving than the $B_1$ and $G_1$ isomers. This could be attributed to the presence of an oleifinic bonds between $C_2$ and $C_3$ of the $B_1$ and $G_1$ components which are readly affected by heat and pressure, while $B_2$ and $G_2$ lacks these oleifinic bonds (TRAGER & STOLOGG, 1967).

| Samples | Dilution-fold induced inhibition | Inhibition zone (mm) | Dilution-fold inducing hatchability | Percentage hatchability |
|---|---|---|---|---|
| Uninfected peanut sample (control) | concentrate | (-)ve | concentrate | 100 |
| Untreated contaminated sample | 3 | 17 | 60 | 40 |
| Roasted sample at 60° C : | | | | |
| 1 h | 3 | 17 | 60 | 40 |
| 2 h | 3 | 17 | 60 | 50 |
| 3 h | 3 | 16 | 60 | 50 |
| at 80° C : | | | | |
| 1 h | 2 | 18 | 55 | 40 |
| 2 h | 2 | 16 | 50 | 50 |
| 3 h | 1,5 | 18 | 40 | 50 |
| at 105° C : | | | | |
| 1 h | 2 | 16 | 50 | 50 |
| 2 h | 1,5 | 18 | 35 | 30 |
| 3 h | 1,5 | [illegible] | 30 | 50 |
| Autoclaved sample at 1.5 atmosphere : | | | | |
| 30 mn | concentrate | 16 | 20 | 50 |
| 60 mn | concentrate | (-)ve | 2 | 50 |
| 90 mn | concentrate | (-)ve | concentrate | 100 |

Table 3. – Dilutions of concentrate extracts of different treatment inducing 50% hatchability of Leghorn eggs (0,02 ml of each dilution) different dilutions and inhibition zone of *B. megaterium* ranged between 8 to 18mm (equal to 0.14 and 1.4 $\mu$ g/disk).

The 20-fold dilution of treated peanuts autoclaved for 30 mn yielded 50% hatchability, whereas the 2-fold dilution of the sample treated for 60 mn yield the same.

From the data of TLC (tab. 1 and 2), chick embryo assay and microbiological test (tab. 3), it can be concluded that aflatoxins are resistant to dry heat, while moist heat when combined with high pressure provides a very effective and satisfactory method to inactive aflatoxins toxicity. Since the treated crushed peanuts does not have a residual smell or taste, it can be spray-dried and used.

ACKNOWLEDGEMENT

The authors are very grateful to Dr. Leo GOLDBLATT, Southern Utilization and Development Division, New Orleans, U.S. for kindly supplying aflatoxins standards.

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